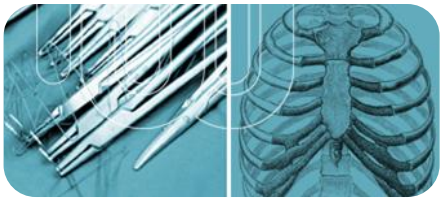


Diploma Thesis

MEDIZINISCHE UNIVERSITÄT WIEN

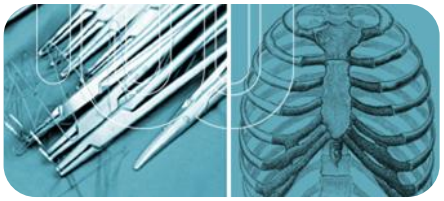
Lymphangiogenesis in Bronchiolitis Obliterans Syndrome

Denise Traxler-Weidenauer

Advisor:
Priv.-Doz. Dr. Konrad HOETZENECKER, PhD

Co-advisor:
Dr. med. univ. Thomas SCHWEIGER

University Clinic of Surgery, Division of Thoracic Surgery,
Medical University of Vienna



Background

Bronchiolitis Obliterans Syndrome

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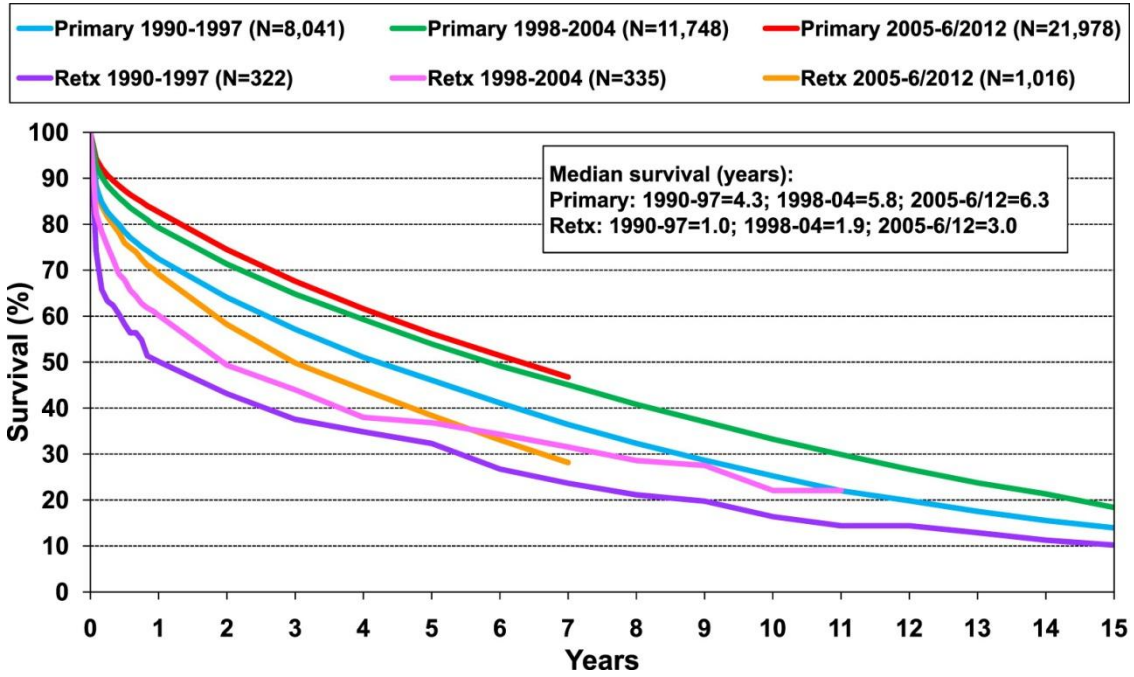

YUSEN RD (2014). The Registry of the International Society for Heart and Lung Transplantation: Thirty-first Adult Lung and Heart–Lung TransplantReport—2014; Focus Theme: Retransplantation

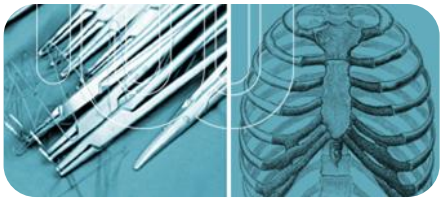

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Table 4 Cumulative Morbidity Rates in Adult Primary Lung Transplant and Lung Retransplant Survivors (Follow-ups: April 1994 to June 2013)

	Within 1 and 5 Years[a] after primary lung transplant				Within 1 and 5 years[a] after lung retransplant[b]			
Outcome	Within 1 year	Total with known response (*n*)	Within 5 years	Total with known response (*n*)	Within 1 year	Total with known response (*n*)	Within 5 years	Total with known response (*n*)
Hypertension	51.5%	15,962	81.6%	4,779	58.1%	516	83.9%	87
Renal dysfunction	22.9%	18,269	54.6%	6,132	27.2%	604	61.6%	146
Abnormal creatinine ≤2.5 mg/dl	16.0%		36.4%		16.4%		34.9%	
Abnormal creatinine >2.5 mg/dl	5.1%		14.5%		7.0%		16.4%	
Chronic dialysis	1.7%		3.0%		3.1%		7.5%	
Renal transplant	0.1%		0.7%		0.7%		2.7%	
Hyperlipidemia	25.8%	16,653	58.7%	5,122	26.6%	541	59.0%	100
Diabetes	23.9%	18,206	40.2%	6,037	22.8%	601	39.9%	138
Bronchiolitis obliterans syndrome	9.2%	17,185	40.3%	5,210	16.7%	568	53.4%	118

[a]Percentage of patients with known responses who had various morbidities as reported on the 1- and 5-year annual follow-up forms post-transplantation. To reduce bias, for 5-year rates, the table only includes patients with responses reported for every follow-up through the 5-year annual follow-up.
[b]Retransplantation refers to the first lung retransplant for lung or heart–lung primary transplant recipients. Retransplant events were identified by a prior transplant reported to the Registry. Because identification of all transplants for an individual may not be complete, the number of retransplant events may be slightly underestimated.

BOS is defined as a progressive airflow obstruction that cannot be explained by acute rejection, infection or other confounding complications.

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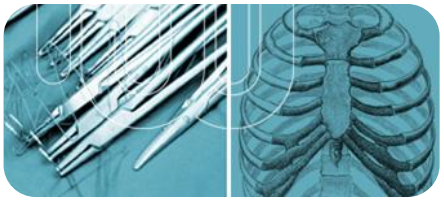

Background

Bronchiolitis Obliterans Syndrome

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Pathogenesis is still obscure, a major role of the **adaptive immune system** is indicated via both **alloimmune** & **non-alloimmune** mechanisms

disruption of the balance between type 1, 2, 17 and Treg immune response

alloimmune reactivity driven by HLA mismatch

humoral immunity

autoimmunity

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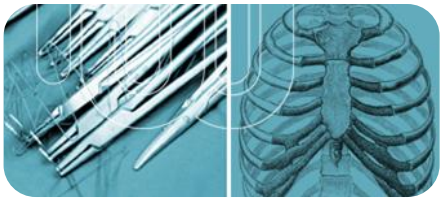

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alloimmune reactivity driven by HLA mismatch
humoral immunity
autoimmunity

Risk factors

acute rejection
CMV pneumonitis/infection
HLA mismatching
primary graft dysfunction
aspiration
lymphocytic bronchitis/bronchiolitis
EBV reactivation
prolonged allograft ischemia

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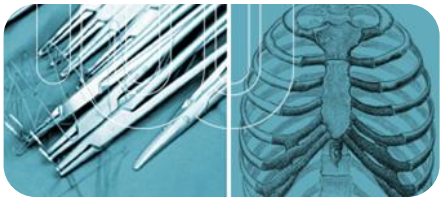

Background

Bronchiolitis Obliterans Syndrome

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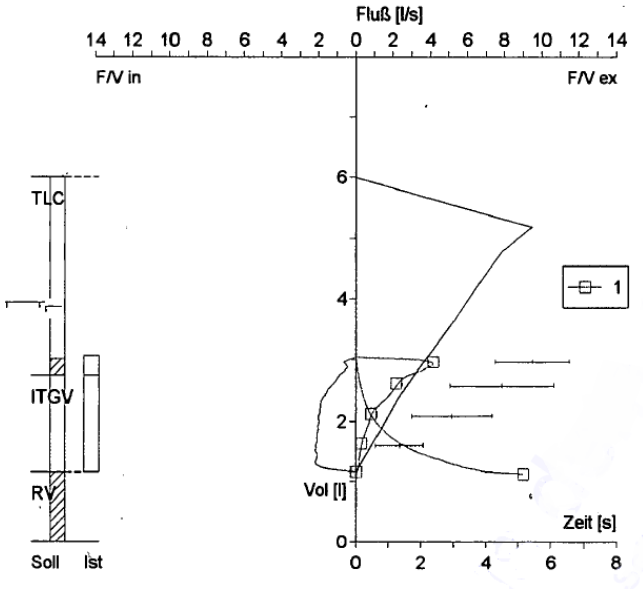
Diagnosis is usually made by **clinical**, physiological and radiographic parameters

2001 Classification according to the ISHLT	
BOS 0	FEV_1 > 90% of baseline and FEF_{25-75} > 75% of baseline
BOS 0-p	FEV_1 81 – 90% of baseline and/or FEF_{25-75} ≤ 75% of baseline
BOS I	FEV_1 66 – 80% of baseline
BOS II	FEV_1 51 – 65% of baseline
BOS III	FEV_1 < 50% of baseline



sustained (≥ 3 weeks) decline of FEV_1 and other possible causes resulting in this decline are excluded

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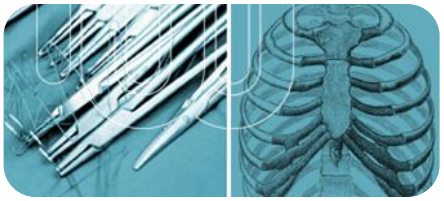

Background

Bronchiolitis Obliterans Syndrome

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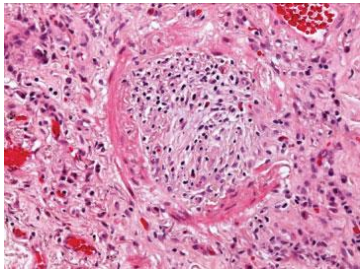
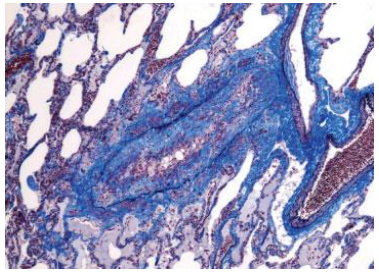
TBBx does not depict a sufficient method for diagnosis





obliterative bronchiolitis characterised by *subepithelial fibrosis* resulting in luminal occlusion, *atrophy of smooth muscle*, *destruction of the elastic part* of the airway wall are is rarely seen

mostly *mucostasis* or *foamy histiocytes* may be seen

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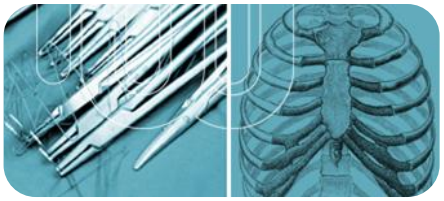

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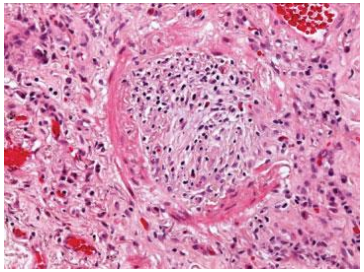

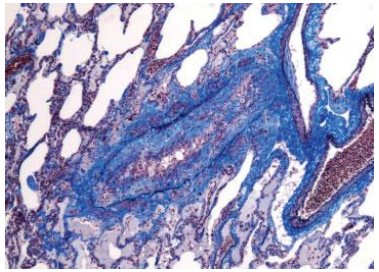

obliterative bronchiolitis characterised by *subepithelial fibrosis* resulting in luminal occlusion, *atrophy of smooth muscle*, *destruction of the elastic part* of the airway wall are is rarely seen
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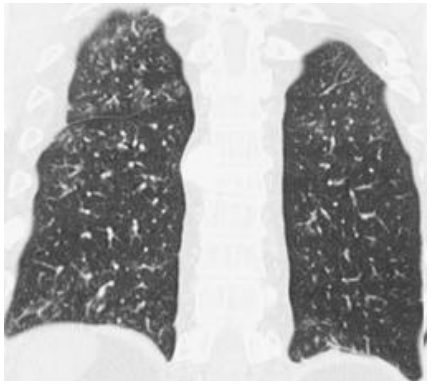
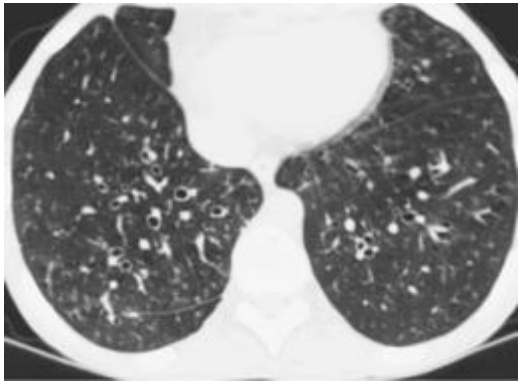
Radiological hallmarks

Decreased attenuation and vascularity

Mosaic perfusion pattern

Air trapping at expiratory images





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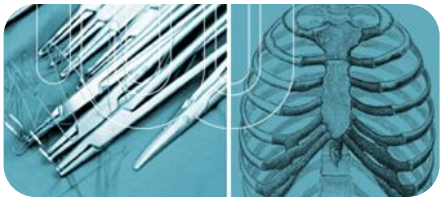

Background

Bronchiolitis Obliterans Syndrome

MEDIZINISCHE UNIVERSITÄT WIEN

Treatment options are disappointing, a stabilisation or reduction of decline in FEV_1, but rarely an improvement has been documented

- adjustment in immunosuppressive therapy
- azithromycin
- extracorporeal photopheresis
- retransplantation

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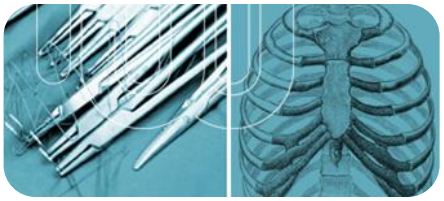

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- adjustment in immunosuppressive therapy
- azithromycin
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By the time BOS is diagnosed irreversible damage to the airways has occurred so that the key to increasing survival is successful **prevention** by e.g. reduction of risk factors and regular follow-up visits.

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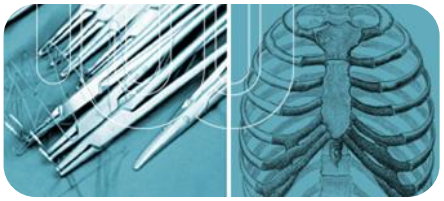

Background

Lymphangiogenesis

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Lymphangiogenesis, the growth of new lymphatic vessels, occurs in physiological & pathological processes in both developmental states & adult individuals

- tissue inflammation
- transplant rejection
- tumour metastases
- wound healing
- development of the corpus luteum

Lymphatic vessels display the afferent arm of the lymphatic system

Many similarities with the blood vascular system

Present in all vascularised tissues except for bone marrow, retina & CNS

Crucially involved in

- regulation of tissue fluid
- immune defence
- absorption and transportation of triglycerides & lipophilic compound

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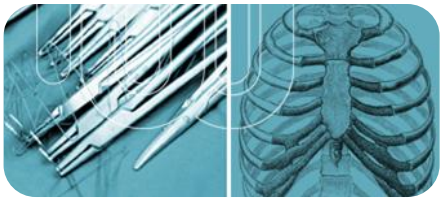
TAMMELA T (2010). Lymphangiogenesis, Molecular mechanisms and future promise.
DIETRICH T (2009). Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation.
OLIVER G (2010). Endothelial cell plasticity: how to become and remain a lymphatic endothelial cell.
JELTSCH M (2003). Genesis and pathogenesis of lymphatic vessels



Background

Lymphangiogenesis

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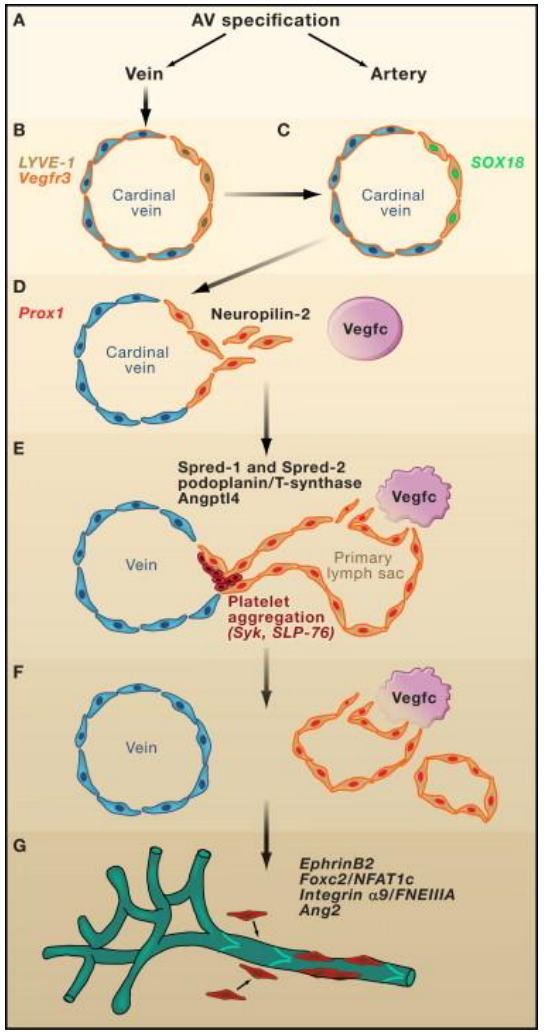
Centrifugal theory of the embryologic development of lymphatic vessels

originating from embryonic veins

at embryonic week 6-7

several key regulators in embryonic development are also involved in adult lymphangiogenesis



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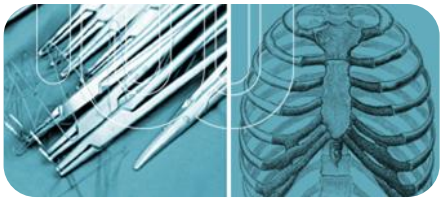

Background

Lymphangiogenesis

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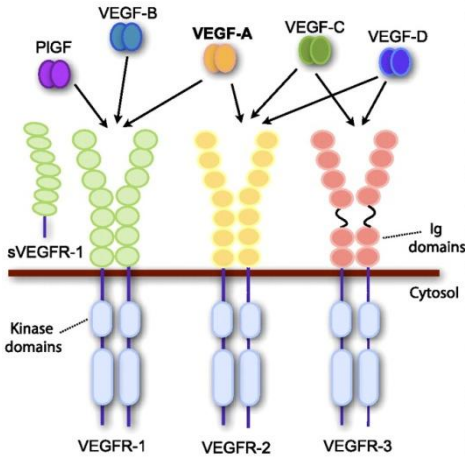
Molecular mechanisms in lymphangiogenesis

VEGFR-3/VEGF-C/-D Axis

proliferation, migration & survival of lymphatic endothelial cells

overexpression of VEGF-C/-D stimulates lymphangio-genesis (e.g. released by macrophages)



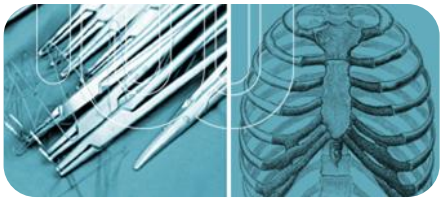
Prox-1 – the master regulator of lymphatic cell fate

Podoplanin – crucial in separation of lymphatic vessels from veins

LYVE-1 – involved transportation of leukocytes throughout lymphatics

All can serve as specific **markers for lymphatic vessels**

RUIZ DE ALMODOVAR C (2009). Role and Therapeutic Potential of VEGF in the Nervous System
TAMMELA T (2010). Lymphangiogenesis, Molecular mechanisms and future promise.
ABTAHIAN F (2003). Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk.
JACKSON DG (2001). LYVE-1, the lymphatic system and tumor lymphangiogenesis.



Background

Lymphangiogenesis in Transplantation

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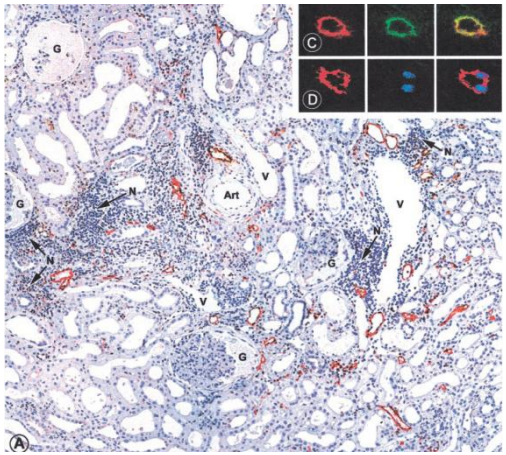
It is suggested that lymphatic vessels are involved in the pathogenesis of graft rejection by **presenting antigens**, **trafficking immune response** and **regulation of fluid homeostasis** and **tissue edema** in either a beneficial or harmful way.

Previous results in…

renal transplantation: increase of lymphatic vessels in kidney grafts – *indicator for superior outcome?*

controversial role in acute rejection – *possible exit route for mononuclear cells?*



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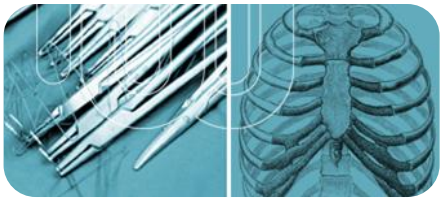

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corneal transplantation: experimental corneal transplantation as a model for allogeneic transplantation
inhibition of lymphangiogenesis induces alleviation of graft rejection

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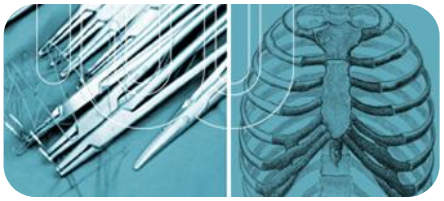

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Lymphangiogenesis in Transplantation

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liver transplantation: lymphangiogenesis is induced in acute rejection - *involvement in resolution?*

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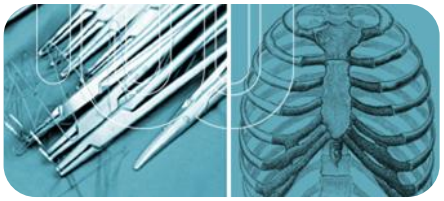

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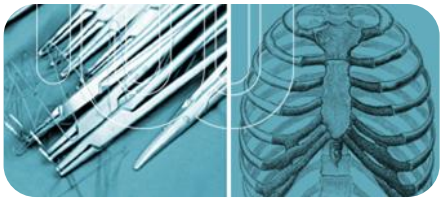

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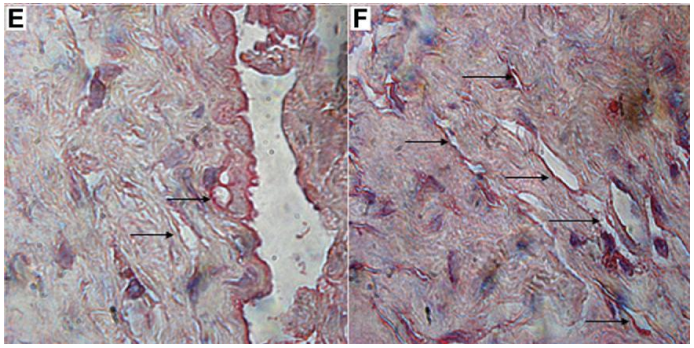
heart transplantation: increased lymphatic vessels density in severe acute rejection

lung transplantation: increased LVD in acute rejection
induction of lymphangiogenesis in a rat model for obliterative airway disease in a VEGF-C dependent manner, inhibition by Cyclosporin A



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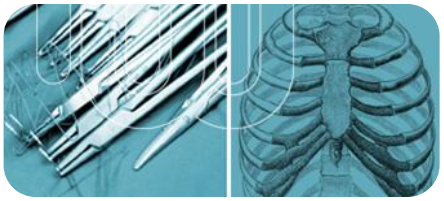

Aims of the Study

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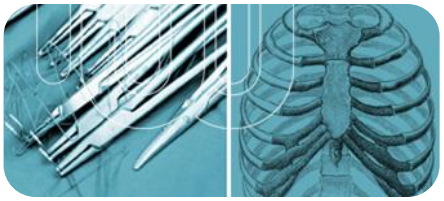
Evaluation of podoplanin positive lymphatic vessels indicating lymphangiogenesis in BOS patients and control subjects

→ increased lymphangiogenesis in BOS patients?

→ correlation of lymphatic vessels with time to BOS III diagnosis?



Material & Methods

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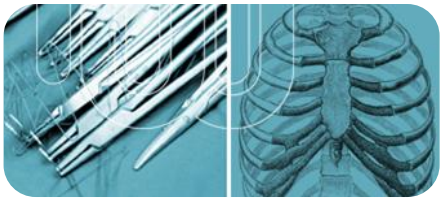
Study collective

36 patients

- 23 BOS patients
- 13 control subjects

Inclusion Criteria

BOS patients	Control subjects
retransplantation at the Division of Thoracic Surgery, MUW	Surgery at the Division of Thoracic Surgery, MUW
BOS as indication of retransplantation	healthy lung tissue could be obtained from this surgical operation
verification of BOS by lung function testing	confirmation by a pathologist
sufficient peribronchial tissue	



Material & Methods

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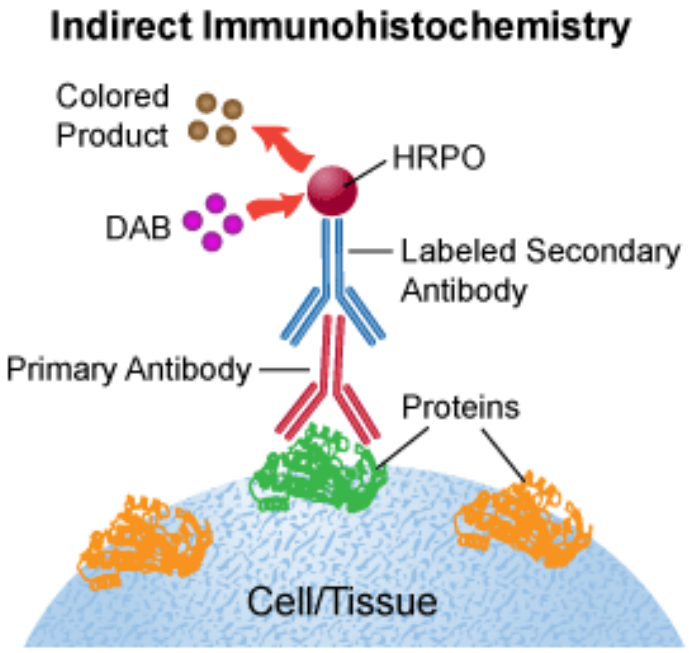
Immunolabelling of lymphatic vessels via immunohistochemistry with ***podoplanin*** as a marker for lymphatic epithelium



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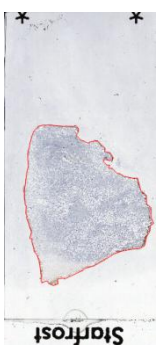
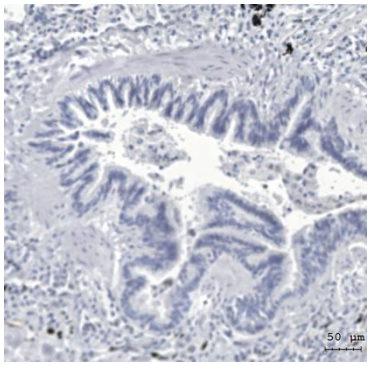
http://www.leinco.com/immunohistochemistry (July 24th, 2015)

TissueFAXS

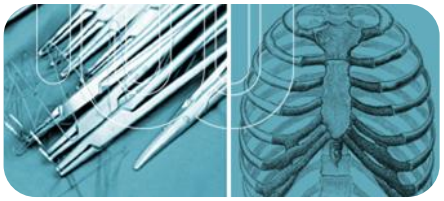
automatic scanning of a whole slide

selection of regions of interests with TissueFAXS Viewer





http://www.tissuegnostics.com/EN/systems/tissuefaxs.php (July 24th, 2015)



Material & Methods

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Evaluation of regions of interest

→ Lymphatic vessels per bronchiole

→ Lymphatic vessels per mm bronchial epithelium

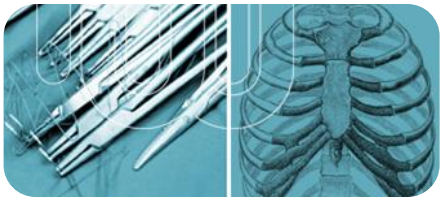
→ µm lymphatic endothelium per mm bronchial epithelium

→ Correlation of lymphatic vessels with time to BOS III diagnosis

Statistics

→ SPSS Statistics 21

→ GraphPad Prism 5



Results

Demographics

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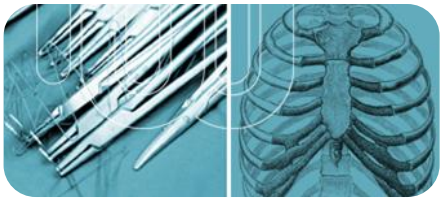
	BOS	Control	p-value
n	23	13	
Presence of infiltrates (with/without)	15/20	3/13	0.0946
Sex (ma/fe) (%)	12 (52) / 11 (48)	7 (54) / 6 (46)	0.923
Diagnosis (%)			
α1-AT deficiency	1 (4)		
Bronchiectasis	1 (4)		
COPD	5 (22)		
CTEPH	1 (4)		
Cystic fibrosis	8 (35)		
LAM	1 (4)		
Pulmonary hypertension	1 (4)		
Pulmonary fibrosis	5 (22)		
LuTX type (%)			
DLuTX	17 (74)		
SLuTX	6 (26)		
Mean ischemic time, min (mean ± SD)	303 ± 74		
ECMO bridging to transplantation (y/n) (%)	2 (11) / 16 (89)		
Intraoperative ECMO support (y/n) (%)	10 (59) / 7 (41)		
Mechanical ventilation, d (mean ± SD)	3 ± 2		
ICU stay, d (mean ± SD)	8 ± 5		
Hospital stay, d (mean ± SD)	24 ± 11		
Immunosuppression			
Cyclosporine A (y/n)	8/13		
Mycophenolic acid (y/n)	19/2		
Tacrolimus (y/n)	13/8		
Azathioprine (y/n)	2/19		
Time to ReTX, m (mean ± SD)	84.13 ± 51.76		
Time to BOS III diagnosis, m (mean ± SD)	62.65 ± 45.28		



Results

Lymphatic Vessel Density

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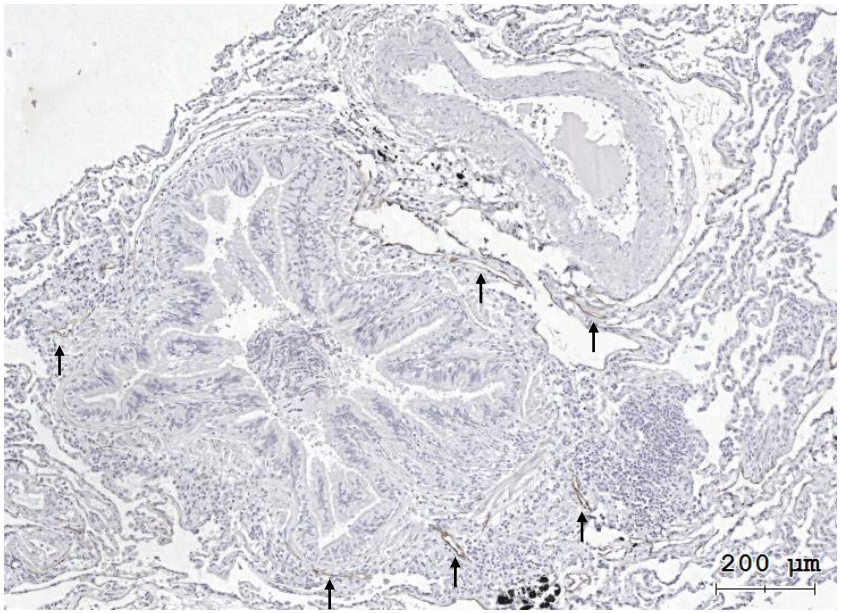
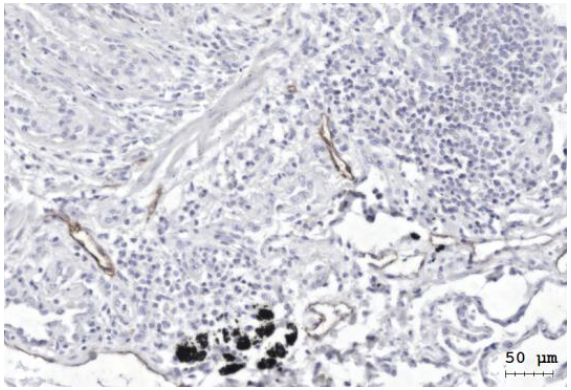
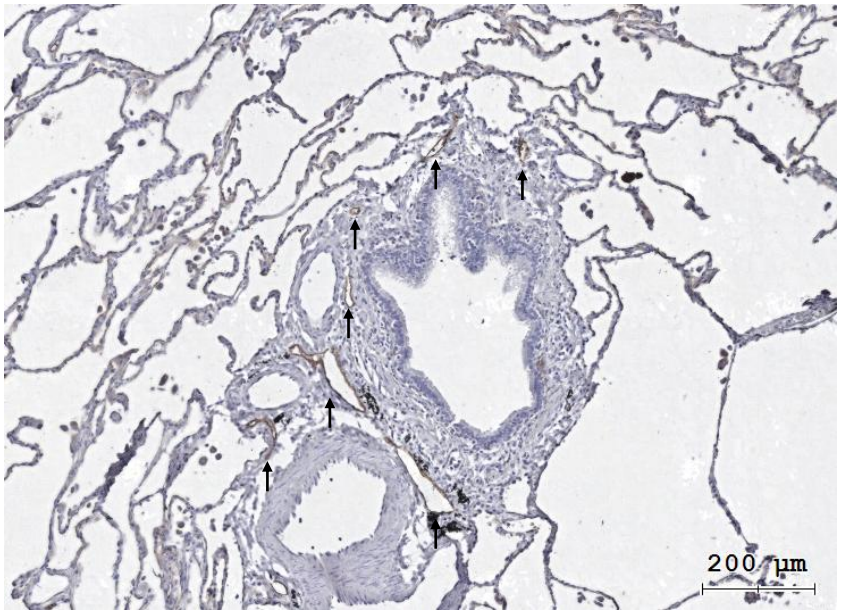
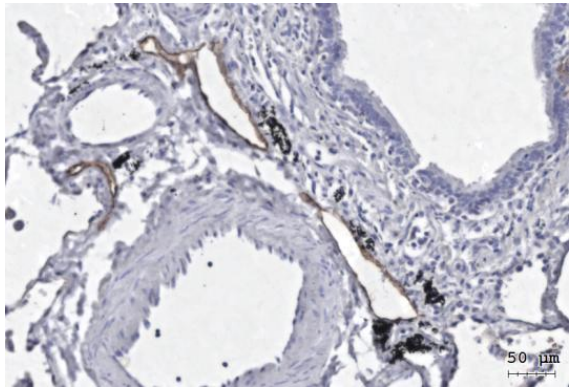
BOS

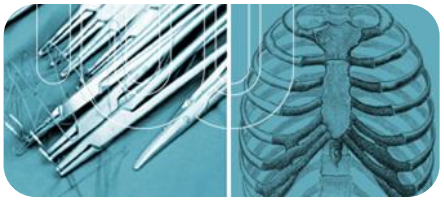




Control







Results

Lymphatic Vessel Density

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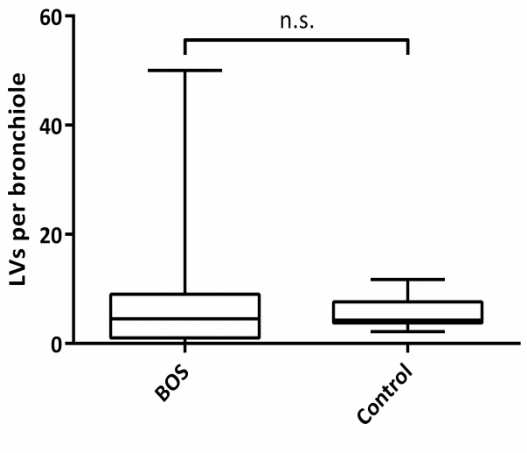
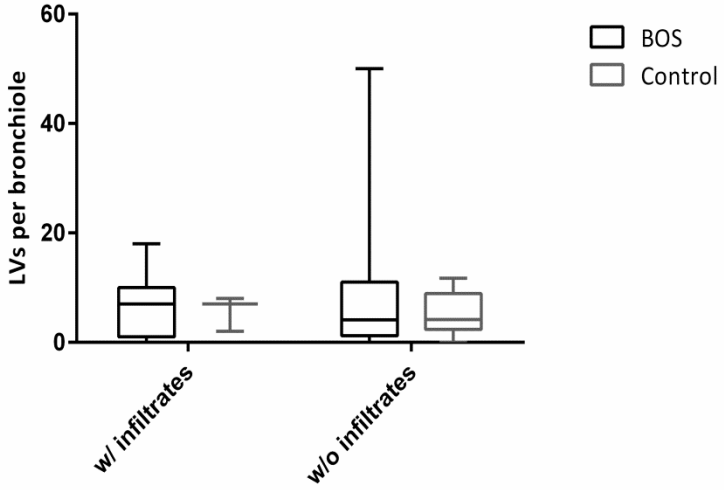
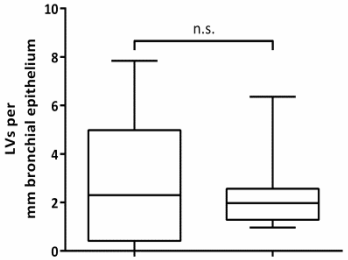
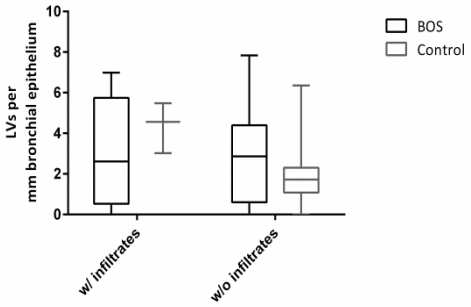
Lymphatic vessels per bronchiole



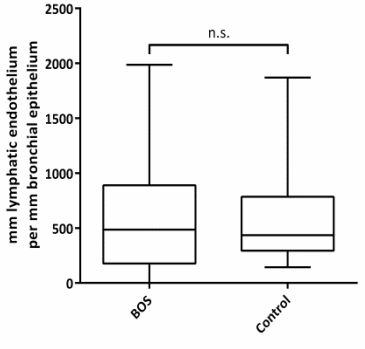


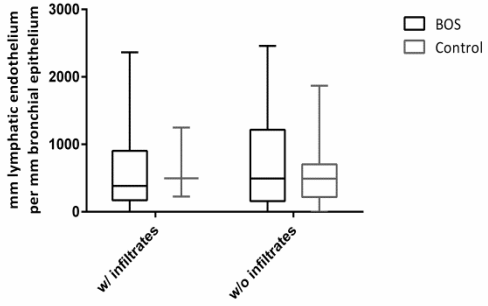
Lymphatic vessels per mm bronchial epithelium

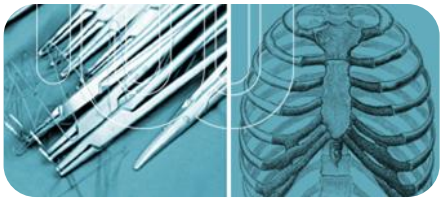




µm lymphatic endothelium per mm bronchial epithelium







Results

Lymphatic Vessel Density

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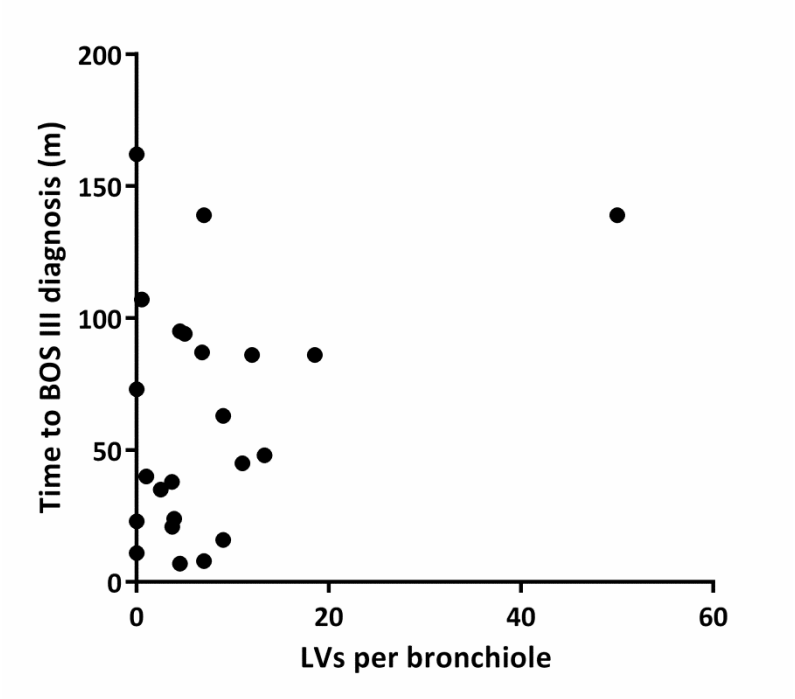
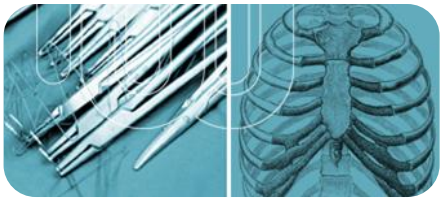
Correlation of LVs per bronchiole with time to BOS III diagnosis





Conclusion

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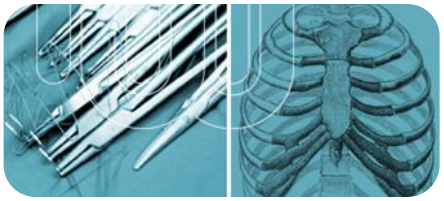
Lymphangiogenesis does not seem to play a role in bronchiolitis obliterans syndrome

increase of lymphatic vessels found in acute rejection of lung allografts at day 14, however no further increase at day 90 – formed lymphatic vessels are already sufficient

equal distribution of inflammatory infiltrates in both groups may indicate a similar inflammatory situation

Cyclosporin features a inhibitory effect on VEGF-C and leads to a decrease of LYVE-1^{+} cells in a obliterative bronchiolitis rat model – patients in this study have received Cyclosporin A



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Thank you for your attention!